

AMENDMENT

To: Examiner of the Patent Office

1. Identification of International Application:

PCT/JP2004/002353

2. Applicant:

Name: Incorporated Administrative Agency National  
Agriculture and Bio-oriented Research Organization

Address: 1-1, Kannondai 3-chome, Tsukuba-shi, Ibaraki  
305-8517 JAPAN

Country of nationality: JAPAN

Country of residence: JAPAN

3. Agent:

Name: 7407 KUBOTA Fujio (Patent Attorney)

Name: 8622 YANO Hiroya (Patent Attorney)

Address: E-1 Bldg., 3-12, Nihombashi 3-chome,  
Chuo-ku, Tokyo 103-0027 JAPAN

4. Item to be Amended: Description, Claims and Drawings

5. Subject Matter of Amendment:

(1) The phrase "when the seed of wheat or barley is impregnated in water, germination is induced to allow the reserve proteins to be decomposed by endogenous protease to thereby increase the free amino acid content in the seed, and that" which appears on page 3, lines 10-12 of the description (i.e. page 4, line 25 - page 5, line 4 of the English translation of description) should be deleted.

(2) The phrase ", and seed subjected to an impregnation treatment in water" which appears on page 3, lines 12-13 of the description (i.e. page 5, lines 5-6 of the English translation of description) should be deleted.

(3) The paragraphs "The invention ... 72 hours." which appears on page 3, lines 17-24 of the description (i.e. page

5, lines 13-23 of the English translation of description) should be deleted.

(4) The words "claim 3" which appears on page 3, line 25 of the description (i.e. page 5, line 24 of the English translation of description) should be amended as --claim 1--.

(5) The words "claim 4" which appears on page 4, line 6 of the description (i.e. page 6, line 8 of the English translation of description) should be amended as --claim 2--.

(6) The words "claim 5" which appears on page 4, line 12 of the description (i.e. page 6, line 16 of the English translation of description) should be amended as --claim 3--.

(7) The words "claim 6" which appears on page 4, line 18 of the description (i.e. page 6, line 24 of the English translation of description) should be amended as --claim 4--.

(8) The words "claims 3 to 5" which appears on page 4, line 20 of the description (i.e. page 6, line 25 - page 7, line 1 of the English translation of description) should be amended as --claims 1 to 3--.

(9) The words "claim 7" which appears on page 4, line 22 of the description (i.e. page 7, line 5 of the English translation of description) should be amended as --claim 5--.

(10) The words "claim 6" which appears on page 4, line 24 of the description (i.e. page 7, line 6 of the English translation of description) should be amended as --claim 4--.

(11) The paragraphs "Fig. 1 is ... arginine content of whole grain flour obtained by subjecting a wheat seed to an impregnation treatment in water." which appears on page 5, lines 11-20 of the description (i.e. page 8, lines 4-18 of the English translation of description) should be deleted.

(12) The paragraphs "The invention ... Also," which appears on page 6, lines 11-25 of the description (i.e. page 9, line 18 - page 10, line 14 of the English translation of description) should be deleted.

(13) The words "claim 3" which appears on page 7, line 6 of the description (i.e. page 10, line 25 of the English translation of description) should be amended as --claim 1--.

(14) The words "claim 4" which appears on page 7, line

12 of the description (i.e. page 11, line 8 of the English translation of description) should be amended as --claim 2--.

(15) The words "claim 5" which appears on page 7, line 18 of the description (i.e. page 11, line 16 of the English translation of description) should be amended as --claim 3--.

(16) The words "claim 6" which appears on page 7, line 24 of the description (i.e. page 11, line 25 of the English translation of description) should be amended as --claim 4--.

(17) The sentences "As the first ... Further," which appears on page 8, lines 18-20 of the description (i.e. page 13, lines 10-13 of the English translation of description) should be deleted.

(18) The sentence "When the glutamine content is ... the water-immersion time." which appears on page 9, lines 15-18 of the description (i.e. page 14, lines 19-22 of the English translation of description) should be deleted.

(19) The whole Example 1 bridging page 9 line 21 to page 10 line 24 of the description (i.e. page 15, line 1 - page 16, line 15 of the English translation of description) should be deleted.

(20) The words "Example 2" which appears on page 10, line 25 of the description (i.e. page 16, line 16 of the English translation of description) should be amended as --Example 1--.

(21) The words "Example 3" which appears on page 12, line 5 of the description (i.e. page 18, line 1 of the English translation of description) should be amended as --Example 2--.

(22) The words "Example 2" which appears on page 12, line 6 of the description (i.e. page 18, line 2 of the English translation of description) should be amended as --Example 1--.

(23) The words "Example 4" which appears on page 12, line 11 of the description (i.e. page 18, line 9 of the English translation of description) should be amended as --Example 3--.

(24) The words "Example 2" which appears on page 12, line 12 of the description (i.e. page 18, line 10 of the English translation of description) should be amended as --Example 1--.

(25) The words "Example 5" which appears on page 12, line 17 of the description (i.e. page 18, line 19 of the English

translation of description) should be amended as --Example 4--.

(26) The word "50m" which appears on page 12, line 19 of the description (i.e. page 18, line 22 of the English translation of description) should be amended as --50mM--.

(27) The words "was preferred" bridging page 13, the second to first lines from the bottom of the description (i.e. page 20, line 4 of the English translation of description) should be amended as --was preferred--. (note: this is a grammatical error in Japanese)

(28) The whole Example 6 bridging page 14 line 3 to page 15 line 7 of the description (i.e. page 29, line 9 - page 22, line 1 of the English translation of description) should be deleted.

(29) The words "Example 7" which appears on page 15, line 8 of the description (i.e. page 22, line 2 of the English translation of description) should be amended as --Example 5--.

(30) The words "Example 2" which appears on page 15, line 9 of the description (i.e. page 22, line 3 of the English translation of description) should be amended as --Example 1--.

(31) The words "Table 4" which appears on page 15, line 11 of the description (i.e. page 22, line 7 of the English translation of description) should be amended as --Table 3--.

(32) The words "Table 4 (No. 1)" which appears on page 16, line 1 of the description (i.e. page 23, line 1 of the English translation of description) should be amended as --Table 3 (No. 1)--.

(33) The words "Table 4 (No. 2)" which appears on page 17, line 1 of the description (i.e. page 24, line 1 of the English translation of description) should be amended as --Table 3 (No. 2)--.

(34) The words "Table 4" which appears on page 18, line 1 of the description (i.e. page 25, line 1 of the English translation of description) should be amended as --Table 3--.

(35) The words "Example 8" which appears on page 18, line 14 of the description (i.e. page 25, line 19 of the English translation of description) should be amended as --Example 6--.

(36) The words "Example 2" which appears on page 18, line

15 of the description (i.e. page 25, line 20 of the English translation of description) should be amended as --Example 1--.

(37) The words "Table 5" which appears on page 18, line 16 of the description (i.e. page 25, line 23 of the English translation of description) should be amended as --Table 4--.

(38) The words "Table 5" which appears on page 19, line 1 of the description (i.e. page 26, line 3 of the English translation of description) should be amended as --Table 4--.

(39) The words "Table 5" which appears on page 19, the sixth line from the bottom of the description (i.e. page 26, line 21 of the English translation of description) should be amended as --Table 4--.

(40) The whole Example 9 bridging page 19 the bottom line to page 21 line 5 of the description (i.e. page 26, bottom line - page 28, line 1 of the English translation of description) should be deleted. (According to this amendment, the whole page 20 of Japanese specification should be deleted.)

(41) The words "Example 10" which appears on page 21, line 6 of the description (i.e. page 28, line 12 of the English translation of description) should be amended as --Example 7--.

(42) The words "Example 2" which appears on page 21, line 8 of the description (i.e. page 28, line 15 of the English translation of description) should be amended as --Example 1--.

(43) The words "Table 7" which appears on page 21, line 9 of the description (i.e. page 28, lines 16-17 of the English translation of description) should be amended as --Table 5--.

(44) The words "Table 7 (No. 1)" which appears on page 22, line 1 of the description (i.e. page 29, line 1 of the English translation of description) should be amended as --Table 5 (No.1)--.

(45) The words "Table 7 (No. 2)" which appears on page 23, line 1 of the description (i.e. page 30, line 1 of the English translation of description) should be amended as --Table 5 (No. 2)--.

(46) The words "Table 7 (No. 3)" which appears on page 24, line 1 of the description (i.e. page 31, line 1 of the English translation of description) should be amended as --Table 5 (No.

3) --.

(47) The words "a seed of wheat or barley" which appears on page 24, the sixth line from the bottom of the description (i.e. page 31, 7th to 6th lines from the bottom of the English translation of description) should be amended as --a ground product of a seed of wheat or barley--.

(48) Claims 6 and 7 should be deleted.

(49) Claims 1 to 5 should be amended.

(50) Figs. 1 to 5 should be deleted.

6. List of Attached Document:

(1) Replacement sheet of pages 3 to 10, 11 to 19 and 21 to 24 of the specification

(2) Replacement sheet of pages 26-27 of claims

(3) Pages 10, 15 and 20 of the specification were removed by amendment.

(4) Pages 1/5 and 2/5 of the drawings were removed.

## Description

### Food Ingredient Including Enriched Free Amino Acids and Their Production Method

#### Technical Field

The present invention relates to a food ingredient including enriched free amino acids and its production method, and particularly relates to a food ingredient having an elevated content of free glutamine, valine, isoleucine, leucine and arginine in a seed of wheat or barley and the like, and its production method. More particularly, the present invention relates to a food ingredient in which the content of the aforementioned 5 kinds of free amino acids is increased to a predetermined value; a method of the production of a food ingredient, the method comprising elevating the content of the free amino acids in a mature seed (including germinated seed) of wheat or barley by allowing a material seed of wheat or barley to be impregnated in water under a certain condition; and a method of the production of the aforementioned food ingredient by immersing at least one of whole meal, bran, shorts, red dog, or 60% flour of the material seed selected from a group of immature seeds of wheat or barley from immediately after the heading until the maturation, mature seeds, seeds prepared from mature seeds by allowing to be impregnated in water under a certain condition (including germinated seeds), in water under

a certain condition.

#### Background Art

There are four methods of the production of an amino acid, i.e., fermentation methods, enzyme methods, synthesis methods and extraction method. Among these, the fermentation method and the enzyme method are principal methods, and 16 kinds of the amino acids among 20 kinds have been industrially produced by either of these two methods. Their applications are in a wide range of medical drugs as well as foods, cosmetics, feeds and the like.

Sodium glutamate is most frequently utilized among amino acids especially for flavoring. Next, quantities of consumed glycine, alanine are great which are an amino acid used for the purpose of improving the taste and keeping shelf life longer. In recent years, while investigations of physiological functions of amino acids have made advances, physiological roles of individual amino acids have been elucidated (see, "Amino Acid Handbook", published by Institute for Industrial Research, p. 51-59, April 2003). As health consciousness of the consumers grows, beverages and supplements produced with focusing attention to health and nutrition functions of amino acids have been found in the market (see, "Syokuhin to Kaihatsu (Foods and Development)", Vol. 34, No. 10, p. 4-8 and p. 20-22, 1999, published by Reserved CMP Japan Co., Ltd.). Above all, an advantage of ingestion of branched chain amino acids



referred to as BCAAs such as valine, leucine and isoleucine, as well as glutamine and arginine has become common knowledge in fields of sports science (see, 57th Proceedings of Congress of Japanese Society of Nutrition and Food Science, p. 287).

Pharmacological actions of these amino acids are shown in Table 1. BCAAs account for 35% in muscular proteins and are consumed in muscle by exercise. Deficiency of BCAAs may result in muscular fatigue, muscular pain, and muscle strain. In order to solve these problems, it is effective to take BCAAs immediately before the exercise, or within 30 minutes immediately after the exercise, to thereby restore the muscle into the normal state. Hence, muscular pain and fatigue are rapidly cured, and muscle stronger than before damage can be constituted.

Amount of requirement of glutamine from intestinal tract and immunocytes is suddenly elevated upon stress during the exercise. Thus, glutamine concentration in skeletal muscle and liver may be reduced, leading to a variety of impairments such as compromised immunity, deterioration of restoring power, decrease in glycogen and break in balance of body's nitrogen balance.

Under such circumstances, supply of glutamine shall be required. Additionally, arginine exhibits a growth hormone secretagogue action, and an action to accelerate highly decomposing ammonia and to enhance cellular immunity. Growth

hormone also relates to enhancement and restoration of muscle, and is believed to accelerate synthesis of muscular protein even more by supply of arginine.

On the other hand, there are no food containing free BCAAs, and glutamine and arginine in high concentrations in nature, and thus, there is no choice but to depend on supplements for the taking of them. However, production cost of BCAAs, and glutamine and arginine is comparatively extremely high among amino acids. Of these, the most inexpensive L-glutamic acid salt of arginine costs 7,000 yen per kg; valine costs 12,000 to 16,000 yen, and isoleucine costs approximately 18,000 yen. Furthermore, production of glutamine and leucine is restricted to use in medical drugs. In addition, predominant production method of leucine is currently the extraction method which requires high costs.

#### Disclosure of the Invention

The present invention provides, for solving the foregoing problems, a food ingredient having an elevated content of free amino acids such as BCAAs, glutamine and arginine in whole meal of the seed of wheat or barley, and in ground products of the seed of wheat or barley during maturation period from immediately after the heading until the maturation, and its production method.

The present inventors found that when the ground product

of the immature seed and mature seed is immersed in water under a certain condition, the protein is decomposed by the action of protease which is endogenously present in high concentration in bran primarily on the external side of the seed, and shorts including germ and thus, specific amino acids are released in high concentrations. According to such findings, the present invention was accomplished.

The invention according to claim 1 relates to a food ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley during maturation period from immediately after the heading until the maturation, wherein the content of free glutamine is 150 to 405 mg/100g, the content of valine is 190 to 325 mg/100g, the content of isoleucine is 125 to 145 mg/100g, the content of leucine is 350 to 520 mg/100g and the content of arginine is 155 to 260 mg/100g.

The invention according to claim 2 relates to a food ingredient that is a 60% flour obtained by grinding a seed of wheat or barley during maturation period from immediately after the heading until the maturation, wherein the content of free glutamine is 70 to 155 mg/100g, the content of valine is 65 to 125 mg/100g, the content of isoleucine is 30 to 60 mg/100g, the content of leucine is 120 to 175 mg/100g and the content of arginine is 105 to 305 mg/100g.

The invention according to claim 3 relates to a food

ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley selected from a group of wheat, malting barley and naked barley, wherein the content of free glutamine is 20 to 430 mg/100g, the content of valine is 20 to 435 mg/100g, the content of isoleucine is 15 to 130 mg/100g, the content of leucine is 35 to 435 mg/100g and the content of arginine is 25 to 300 mg/100g.

The invention according to claim 4 is a method of the production of the food ingredient according to any one of claims 1 to 3 which comprises allowing a ground product of a seed of wheat or barley selected from a group of wheat, malting barley and naked barley to be immersed in water under a condition of a pH of 3.0 to 5.5 and at 40 to 60°C for 1 to 6 hours.

The invention according to claim 5 is the method of the production of the food ingredient according to claim 4 wherein the ground product of the seed of wheat or barley is at least one selected from a group of whole meal, bran, shorts, red dog, and 60% flour.

According to the invention, a food ingredient having the elevated free BCAAs, glutamine and arginine content can be produced by a simple operation from at least one of whole meal, bran, shorts, red dog, and 60% flour of a seed of wheat or barley selected from a group of wheat, malting barley and naked barley. It is believed that a novel demand can be raised by producing the food ingredient according to the invention, also for bran,

shorts and red dog the use of which had been conventionally restricted to feeds and sources of cooking oil, and immature seeds and seeds subjected to an impregnation treatment in water (including germinated seeds) which had not been utilized in cooking ingredients, as well as sprouting seeds having a deteriorated commercial value.

The BCAAs, glutamine and arginine content are elevated in the food ingredient according to the invention, therefore, it is effective in enhancement of basic physical fitness, strengthening of muscle force, relief of fatigue and the like.

#### Brief Description of the Drawings

Fig. 6 is a view showing an optimum pH of the amino acids produced by a water-immersion treatment of whole meal of a wheat seed.

Fig. 7 is a view showing an optimum pH of the amino acids produced by a water-immersion treatment of whole meal of a wheat seed.

Fig. 8 is a view showing an optimum temperature of the amino acids produced by a water-immersion treatment of whole meal of a wheat seed.

Fig. 9 is a view showing an optimum temperature of the amino acids produced by a water-immersion treatment of whole meal of a wheat seed.

### Best Mode for Carrying Out the Invention

Although cultivars and breeding lines of the wheat or barley which may be used in the present invention are not limited, cultivars including a large amount of production of the free amino acids (for example, in case of wheat, Esimasinriki, Asakazekomugi, Tikugoizumi and the like) as well as domestically popular cultivars the seeds of which are readily available (Norin 61, Shirasagikomugi, Fukusayaka and the like) are preferably utilized.

In addition to wheat, malting barley, naked barley and the like can be similarly applied to the invention.

According to the invention, a so-called sprouting seed which had overtaken in the rainfall during maturation to lead to germinated state can be used as a raw material. In connection with the degree of maturation of the immature seed, more immature seeds exhibit more excellent ability of producing the amino acids, however, as they are more immature, treatments for threshing, drying and the like shall be complicated due to the higher moisture content and the smaller size. The seeds on 4 to 5 weeks after the heading have comparatively favorable in use/utilization, and the treatments for the harvesting and drying can be readily conducted.

The invention according to claim 1 is a food ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley during maturation period from

immediately after the heading until the maturation, wherein the content of free glutamine is 150 to 405 mg/100g, the content of valine is 190 to 325 mg/100g, the content of isoleucine is 125 to 145 mg/100g, the content of leucine is 350 to 520 mg/100g and the content of arginine is 155 to 260 mg/100g.

Moreover, the invention according to claim 2 is a food ingredient that is a 60% flour obtained by grinding a seed of wheat or barley during maturation period from immediately after the heading until the maturation, wherein the content of free glutamine is 70 to 155 mg/100g, the content of valine is 65 to 125 mg/100g, the content of isoleucine is 30 to 60 mg/100g, the content of leucine is 120 to 175 mg/100g and the content of arginine is 105 to 305 mg/100g.

The invention according to claim 3 is a food ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley selected from a group of wheat, malting barley and naked barley, wherein the content of free glutamine is 20 to 430 mg/100g, the content of valine is 20 to 435 mg/100g, the content of isoleucine is 15 to 130 mg/100g, the content of leucine is 35 to 435 mg/100g and the content of arginine is 25 to 300 mg/100g.

These food ingredients can be produced in accordance with the invention according to claim 4. More specifically, in the method, a ground product of a seed of wheat or barley selected from a group of wheat, malting barley and naked barley

(including seed of wheat or barley during maturation) is allowed to be immersed in water under a condition of a pH of 3.0 to 5.5 and at 40 to 60°C for 1 to 6 hours. Separation of the wheat or barley such as wheat flour may be carried out by milling of the seed with a flour mill (for example, manufactured by Buhler K.K., Buhler test mill or the like). Depending on gap size between the rolls and size of the sieve, the separation can be perfected into: bran including a seed coat as a principal component and having an aleurone layer; shorts including a germ as a principal component but also including the seed coat and the aleurone layer; red dog including the aleurone layer and albumen; and 60% flour including albumen as a principal component. The 60% flour is also referred to as first grade flour or special grade flour, indicating commercially available wheat flour.

A food ingredient in the form of an aqueous solution can be obtained from the ground product of seeds of wheat or barley including enriched free amino acids as described above by eliminating the insoluble matters. Additionally, a powdery food ingredient can be obtained by subjecting the same to a drying treatment at 110°C or lower. When all the reaction products are utilized, a powdery food ingredient can be obtained similarly by subjecting to a drying treatment at 110°C or lower. Furthermore, a food ingredient capable of being promptly processed can be obtained by adjusting the moisture



content through separately adding milled flour to the reaction product as desired. Herein, the ground product of the seed of wheat or barley refers to any one of whole meal, bran, shorts, red dog, 60% flour, or a mixture of two or more thereof.

As described hereinabove, the method of the production of a food ingredient having an elevated amino acid content may be suitably selected depending on the shape of the target wheat or barley. In case of ground product of the seed of wheat or barley selected from a group of wheat, malting barley and naked barley, for example, when the ground product is at least one of whole meal, bran, shorts, red dog, and 60% flour, the reaction may be allowed by adding water having a pH adjusted to 3.0 to 5.5, and preferably 4.0 to 5.0 to the ground product in an amount of 5 to 40 times, followed by reciprocal shaking under a condition of at 40 to 60°C, usually at 45 to 55°C, at 50 to 150 rpm for 30 minutes or longer, usually for 1 to 6 hours, preferably at 45°C and at 80 to 120 rpm for 1 to 6 hours.

The acid for use in adjusting the pH may be any one of organic acids and inorganic acids. Preferably, an organic acid such as acetic acid, citric acid or ascorbic acid, or an inorganic acid such as hydrochloric acid, sulfuric acid or phosphoric acid may be used. Also, examples of alkali which may be used include sodium phosphate, potassium phosphate, sodium carbonate, sodium hydroxide and the like.

The food ingredient of the invention enriched with free

amino acids including the ground product of the seed of wheat or barley can contribute to enhancement of basic physical fitness, strengthening of muscle force, relief of fatigue and the like by routinely taking the food ingredient. In this instance, when it is taken, for example, immediately after the exercise, it is desired that the composition ratio of each amino acid of valine, isoleucine, leucine, arginine and glutamine is regulated to be 1:1:1-2:1 or greater:1 or greater.

Moreover, although the intake needed a dosage may be dependent on physical activity, generally, rough standard may be approximately 500 to 2000 mg in total amount of BCAAs.

Next, the present invention will be explained in more detail by way of Examples, however, the invention is not limited thereto.

#### Example 1

To 0.2 g of bran, shorts, red dog, and 60% flour prepared from a mature seed of wheat (Fukusayaka) was added 4 ml of 50 mM potassium phosphate buffer (pH 4.5). The mixture was shaken at 45°C and at 100 rpm. Thus, free glutamine, valine, isoleucine, leucine and arginine can be produced. Table 1 shows the amount of amino acids produced by the reaction for 2 hours. The value in parentheses indicates the initial content. Total amount following the reaction is a sum of the amount of production by the reaction and the initial content.

Table 1

	Amount of production (mg/100g)			
	Bran	Shorts	Red dog	60% flour
Glutamine	59.26 (0.00)	45.82 (0.00)	0.29 (0.00)	0.72 (0.00)
Valine	46.45 (5.67)	73.05 (6.33)	9.39 (1.69)	0.58 (1.40)
Isoleucine	24.10 (4.11)	39.02 (4.68)	5.03 (2.02)	2.31 (1.95)
Leucine	87.43 (5.25)	127.5 (5.66)	22.93 (2.58)	3.92 (1.15)
Arginine	54.91 (26.98)	78.07 (49.45)	10.63 (8.77)	11.34 (4.39)

As is clear from Table, the amount of production of glutamine was the largest in bran, while the amount of production of the amino acids other than that was the largest in shorts. The amount of production in shorts was large in the order of leucine, arginine, valine, glutamine and isoleucine, while that in bran followed the order of leucine, glutamine, arginine, valine and isoleucine. In red dog and 60% flour, the amount of production was less than that in the bran and shorts. It was characterized in that the production of glutamine is low, while production of leucine and arginine is comparatively high.

#### Example 2

According to Example 1, using the whole meal of Fukusayaka, a treatment was carried out under a condition of at 40°C, a pH of 3.0 to 5.5 for 1 hour, and the amount of production of each amino acid was measured. Consequently, as shown in Fig. 6 and Fig. 7, the production of glutamine and valine was optimum at a pH of 4.0, while the production of isoleucine, leucine and arginine was optimum at a pH of 4.5.

#### Example 3

According to Example 1, using the whole meal of Fukusayaka, an immersion treatment in water was carried out under a condition of at 10 to 70°C, a pH of 4.5 for 1 hour, and the amount of production of each amino acid was measured. Consequently, as shown in Fig. 8 and Fig. 9, the production of glutamine and arginine was optimum at 45°C, while the production of valine, isoleucine and leucine was optimum at 50°C. When the temperature was 25°C or lower and 60°C or higher, the amount of production was drastically decreased.

#### Example 4

An immature seed of Tikugoizumi 4 week after the heading was freeze-dried, and to 0.1 g of the mixture of bran and shorts and 60% flour of the dry seed was added 4 ml of 50 mM potassium phosphate buffer (pH 4.5). The mixture was shaken at 45°C and at 100 rpm. Thereafter, the amount of thus produced free amino acid was measured. The results are shown in Table 2.

Table 2

Amount of production (mg/100g)								
	The mixture of bran and shorts				60% Flour			
	initial value	1 hour	4 hrs	6 hrs	initial value	1 hour	4 hrs	6 hrs
Glutamine	160.72	123.5	243.2	98.46	66.08	70.08	97.43	47.07
Valine	110.84	121.7	242.7	222.47	80.20	38.18	100.2	62.76
Isoleucine	56.64	53.49	126.3	87.71	40.32	16.30	46.12	33.81
Leucine	84.20	192.0	450.9	445.78	58.24	52.67	156.2	131.77
Arginine	53.96	111.7	245.0	234.64	28.36	25.50	78.32	54.43

As shown in Table 2, initial content of each amino acid was high in the ground product of the immature seed, and the amount of production of each amino acid by the reaction was also extremely large. In the mixture of bran and shorts, all amino acids other than isoleucine were produced in an amount of 100 mg/100 g or more by the reaction for 1 hour, while all amino acids other than isoleucine were produced in an amount of 200 mg/100 g or more by the reaction for 4 hours. The amount of production in the 60% flour was low in comparison with the mixture of bran and shorts, however, it had an ability of the production which was nearly equal to that of the shorts of the mature seed. To the contrary, when the reaction was over 4 hours, the amount of the amino acid obtained after the reaction began to decrease in both of the mixture of bran and shorts

and 60% flour, and after 8 hours, the amount became nearly equal to the initial value.

Therefore, when the immature seed was allowed to react, the reaction for 1 to 4 hours was preferred. When the composition of the amino acids produced in the bran of the immature seed was compared with that produced in the shorts of the mature seed, the ratio of glutamine was high while the ratio of isoleucine was low in the immature seed.

#### Example 5

According to Example 1, each amino acid was produced by a reaction at 45°C and a pH of 4.5 for 1 hour using whole meal of the seed of each cultivars and breeding lines of wheat. Measurement results of the amount of production of each amino acid are shown in Table 3. The values are presented by a unit of mg/100 g. The value in parentheses indicates the initial content. Total amount after the reaction is a sum of the amount of production by the reaction and the initial content.

Table 3 (No. 1)

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Norin 61	5.88	5.86	7.25	16.85	11.31
	(0.00)	(1.67)	(1.49)	(2.15)	(6.69)
Shirasagikomugi	5.98	8.30	6.88	21.50	12.88
	(0.00)	(1.81)	(1.40)	(1.82)	(7.20)
Chugoku 143	6.35	6.19	5.69	18.24	12.25
	(0.00)	(2.09)	(1.75)	(2.60)	(9.26)
Fukusayaka	7.98	8.08	3.79	22.11	12.02
	(0.00)	(2.57)	(2.76)	(2.23)	(14.92)
Chugoku 147	6.86	6.84	6.42	19.38	11.29
	(0.00)	(2.13)	(1.61)	(2.41)	(7.00)
Chugoku 149	6.66	7.72	5.72	20.28	17.39
	(0.00)	(1.96)	(1.50)	(1.82)	(9.26)
Chugoku 152	7.54	8.12	7.49	23.01	18.12
	(0.00)	(1.77)	(1.45)	(1.97)	(8.33)
Chugoku 140	7.59	12.74	7.60	24.60	22.60
	(0.00)	(0.91)	(1.58)	(2.07)	(6.91)
Norin 17	5.93	10.96	7.31	20.93	18.19
	(0.00)	(0.85)	(1.77)	(2.52)	(7.41)
Produra	26.37	13.29	7.57	20.32	18.98
	(0.00)	(1.15)	(2.45)	(3.26)	(17.29)
Esimasinriki	14.19	21.00	7.31	30.60	21.95
	(0.00)	(1.49)	(2.63)	(3.50)	(7.20)
Shinchunaga	11.57	16.53	8.18	26.48	19.35
	(0.00)	(1.40)	(2.22)	(3.19)	(17.50)
Saikai 180	10.48	12.34	5.66	18.77	13.41
	(0.00)	(1.11)	(1.91)	(2.90)	(21.72)
Haruyutaka	5.42	10.81	5.87	16.08	15.40
	(0.00)	(0.71)	(1.72)	(2.84)	(9.16)

Table 3 (No. 2)

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Coco	6.31	13.21	6.33	22.03	19.88
	(0.00)	(0.93)	(1.63)	(2.13)	(9.94)
Roblin	10.11	14.38	7.37	22.52	24.02
	(0.00)	(0.89)	(1.42)	(1.69)	(10.29)
Kanto 107	8.14	15.83	8.09	23.46	24.34
	(0.00)	(0.74)	(1.45)	(2.02)	(7.31)
Tikugoizumi	12.27	16.13	7.80	24.77	24.49
	(0.00)	(1.01)	(1.72)	(2.67)	(8.34)
Jessore	10.93	17.25	8.06	25.13	26.06
	(0.00)	(1.30)	(1.75)	(2.50)	(7.51)
Nanbukomugi	6.25	13.07	6.91	19.83	15.15
	(0.00)	(1.08)	(1.59)	(2.12)	(15.64)
Toyohokomugi	8.55	10.05	5.95	16.24	21.95
	(0.00)	(0.63)	(1.42)	(2.14)	(5.30)
Asakazekomugi	12.41	18.13	8.55	30.19	26.36
	(0.00)	(0.85)	(1.60)	(1.98)	(8.13)
Minaminokomugi	9.67	18.05	7.98	29.70	29.58
	(0.00)	(0.83)	(1.43)	(2.03)	(11.32)
Fukuhokomugi	11.00	15.91	7.89	27.58	24.19
	(0.00)	(0.90)	(1.41)	(1.85)	(11.33)
1CW	10.12	15.98	6.16	25.95	23.94
	(0.00)	(2.34)	(2.67)	(3.42)	(14.41)
PH	21.69	14.92	4.83	19.50	28.85
	(0.00)	(2.46)	(3.36)	(3.28)	(17.60)
ASW	6.05	11.90	7.02	20.32	13.00
	(0.00)	(1.66)	(2.23)	(2.93)	(5.15)
WW	9.37	12.63	4.42	17.46	16.66
	(0.00)	(2.23)	(2.63)	(3.26)	(9.78)



As shown in Table 3, each amino acid was produced in all cultivars and breeding lines. Amount of production was large for glutamine in the order of Produra, PH, Esimasinriki, Asakazekomugi and Tikugoizumi; for valine in the order of Esimasinriki, Asakazekomugi, Minaminokomugi, Jessore and Shinchunaga; for isoleucine in the order of Asakazekomugi, Shinchunaga, Kanto 107, Jessore and Minaminokomugi; for leucine in the order of Esimasinriki, Asakazekomugi, Minaminokomugi, Fukuhokomugi and Shinchunaga; for arginine in the order of Minaminokomugi, PH, Asakazekomugi, Jessore and Tikugoizumi.

In any of the cultivars, the amount of production of each amino acid increased in a time dependent manner. For example, in case of Fukusayaka, the reaction for 6 hours yielded glutamine, valine, isoleucine, leucine and arginine of 16.26, 17.93, 11.94, 27.70 and 24.17 mg per 100 g, respectively. As the reaction time was prolonged, the amount of production was further increased.

#### Example 6

According to Example 1, each amino acid was produced by a reaction under a condition of a pH of 4.5 and at 45°C for 1 hour using whole meal of the seed of each cultivar of the barleys. Results are shown in Table 4. The values are presented by a unit of mg/100 g. The value in parentheses indicates the initial content. Total amount after the

reaction is a sum of the amount of production by the reaction and the initial content.

Table 4

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Naked barley					
Mantenboshi	3.23 (0.00)	13.03 (8.02)	5.87 (4.48)	20.07 (4.08)	12.90 (7.40)
Ichibanboshi	1.27 (0.00)	12.08 (4.64)	7.86 (2.67)	25.99 (2.32)	17.39 (3.42)
Daishimochi	2.38 (0.00)	12.12 (3.52)	9.39 (1.62)	28.44 (0.99)	15.88 (4.32)
Malting barley					
Nishinochikara	18.32 (38.34)	11.10 (4.78)	5.05 (3.53)	21.34 (4.23)	14.11 (4.76)
Amagi Nijo	16.93 (14.15)	13.58 (4.59)	10.17 (1.87)	32.64 (2.03)	16.56 (4.79)
Skygolden	14.34 (20.71)	11.03 (4.70)	3.96 (3.92)	18.81 (4.87)	15.17 (4.68)

As shown in Table 4, similar to wheat, free amino acids were produced by allowing the milled flour to be immersed in water. In the naked barley, the amount of production was large for amino acids in the order of leucine, arginine, valine, isoleucine and glutamine. To the contrary, in malting barley, the amount followed the order of leucine, glutamine, arginine, valine and isoleucine.

Example 7

Bran and shorts prepared from the seed of each cultivars and breeding lines of wheat were allowed to react at a pH of 4.5 and at 45°C for 1 to 6 hours according to Example 1. The amount of production of the amino acids then is shown in Table 5.

Table 5 (No. 1) Initial value

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Bran					
Norin 61	0.00	3.68	2.22	5.03	12.10
Fukusayaka	0.00	5.67	4.11	5.25	26.98
Produra	0.00	2.54	3.65	7.67	31.27
Esimasinriki	0.00	3.29	3.91	8.24	13.02
Asakazekomugi	0.00	1.88	2.38	4.66	14.70
Minaminokomugi	0.00	1.83	2.13	4.78	20.48
Shorts					
Norin 61	0.00	4.11	2.53	5.46	22.17
Fukusayaka	0.00	6.33	4.68	5.66	49.45
Produra	0.00	2.83	4.15	8.27	57.30
Esimasinriki	0.00	3.67	4.46	8.98	23.86
Asakazekomugi	0.00	2.09	2.71	5.03	26.95
Minaminokomugi	0.00	2.04	2.42	5.15	37.52

Table 5 (No. 2) Amount of production by 1-hour reaction

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Bran					
Norin 61	24.45	19.54	28.12	36.65	27.38
Fukusayaka	33.19	26.94	14.72	48.09	29.10
Produra	109.66	44.31	29.35	44.19	45.95
Esimasinriki	59.01	70.02	28.35	66.15	53.14
Asakazekomugi	51.61	60.45	33.16	65.66	63.82
Minaminokomugi	40.21	60.18	30.95	64.59	71.61
Shorts					
Norin 61	18.57	29.67	44.04	52.49	38.20
Fukusayaka	25.20	40.91	23.02	68.85	40.60
Produra	83.28	67.29	45.98	63.28	64.10
Esimasinriki	44.81	106.32	44.40	95.29	74.13
Asakazekomugi	39.19	91.79	51.94	94.01	89.03
Minaminokomugi	30.54	91.38	48.47	92.49	99.48

Table 5 (No. 3) Amount of production by 6-hours reaction

	Glutamine	Valine	Isoleucine	Leucine	Arginine
Bran					
Norin 61	171.28	116.26	41.38	228.48	113.59
Fukusayaka	123.85	104.05	61.70	181.65	108.72
Produra	430.89	191.20	81.23	223.97	148.13
Esimasinriki	298.86	289.37	73.02	295.10	168.09
Asakazekomugi	271.42	260.84	72.76	298.09	193.27
Minaminokomugi	211.50	265.22	72.33	286.42	212.05
Shorts					
Norin 61	131.17	174.68	66.19	331.57	160.69
Fukusayaka	94.85	156.33	97.72	263.93	153.80
Produra	329.98	287.28	129.98	325.04	209.97
Esimasinriki	228.87	434.76	116.84	428.28	237.78
Asakazekomugi	207.84	391.90	116.42	432.68	273.89
Minaminokomugi	161.94	398.48	115.74	415.88	299.97

### Industrial Applicability

The food ingredient according to the invention is a ground product of a seed of wheat or barley including enriched free BCAAs, glutamine and arginine, and the milled flour thereof, which can be similarly utilized to conventional wheat flour and wheat processed products. For example, it can be utilized in materials of noodles such as Japanese wheat noodles (udon) and silver line noodles (somen), breads, snacks, flour

paste products such as rice cake and dumpling cake made of rice or wheat flour. In case of use in the bread, savor can be improved by adding raisin, walnut, sesame, herb or the like to the material. Moreover, amino acids are dissolved in the water soluble fraction of the milled flour to which an appropriate amount of water was added, and the reaction product obtained by allowing the milled flour to be immersed in water to react, therefore, they can be utilized also in beverages and the like by: a method in which precipitates and insoluble matters are removed, followed by subjecting to a processing such as seasoning and sterilization; a method in which the ground product, particularly green powder harvested earlier is placed in a tea bag, and brewed alone or together with a tea leaf; or the like. In addition, when the liquid matter is subjected to a drying treatment, it can be also used in the form of flour, tablet or the like, as a supplement that is effective in muscular pain, chronic fatigue, and relief of fatigue after exercise.

Claims:

1. (Amended) A food ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley during maturation period from immediately after the heading until the maturation, wherein the content of free glutamine is 150 to 405 mg/100g, the content of valine is 190 to 325 mg/100g, the content of isoleucine is 125 to 145 mg/100g, the content of leucine is 350 to 520 mg/100g and the content of arginine is 155 to 260 mg/100g.

2. (Amended) A food ingredient that is a 60% flour obtained by grinding a seed of wheat or barley during maturation period from immediately after the heading until the maturation, wherein the content of free glutamine is 70 to 155 mg/100g, the content of valine is 65 to 125 mg/100g, the content of isoleucine is 30 to 60 mg/100g, the content of leucine is 120 to 175 mg/100g and the content of arginine is 105 to 305 mg/100g.

3. (Amended) A food ingredient that is a mixture of bran and shorts obtained by grinding a seed of wheat or barley selected from a group of wheat, malting barley and naked barley, wherein the content of free glutamine is 20 to 430 mg/100g, the content of valine is 20 to 435 mg/100g, the content of isoleucine is 15 to 130 mg/100g, the content of leucine is 35 to 435 mg/100g and the content of arginine is 25 to 300 mg/100g.

4. (Amended) A method of the production of the food ingredient according to any one of claims 3 to 5 which comprises

allowing a ground product of a seed of wheat or barley selected from a group of wheat, malting barley and naked barley to be immersed in water under a condition of a pH of 3.0 to 5.5 and at 40 to 60°C for 1 to 6 hours.

5.(Amended) The method of the production of the food ingredient according to claim 6 wherein the ground product of the seed of wheat or barley is at least one selected from a group of whole meal, bran, shorts, red dog, and 60% flour.

6.(Deleted)

7.(Deleted)



### Abstract

The present invention provides a food ingredient including elevated content of free amino acids such as glutamine, valine, isoleucine, leucine and arginine in whole meal of a seed of wheat or barley, ground products of a seed of wheat or barley during maturation period from immediately after the heading until the maturation, and the like, and its production method.

More particularly, a food ingredient that is whole meal of a seed of wheat or barley, wherein the content of free glutamine is 200 to 1200 mg/100g, the content of valine is 40 to 150 mg/100g, the content of isoleucine is 30 to 120 mg/100g, the content of leucine is 40 to 150 mg/100g and the content of arginine is 60 to 150 mg/100g, and a method of the production of the food ingredient which includes allowing a seed of wheat or barley selected from a group of wheat, malting barley and naked barley to be impregnated in water at 20 to 30°C for 24 to 72 hours, and the like are provided.

1 / 7

***FIG. 1***

***FIG. 2***

2 / 7

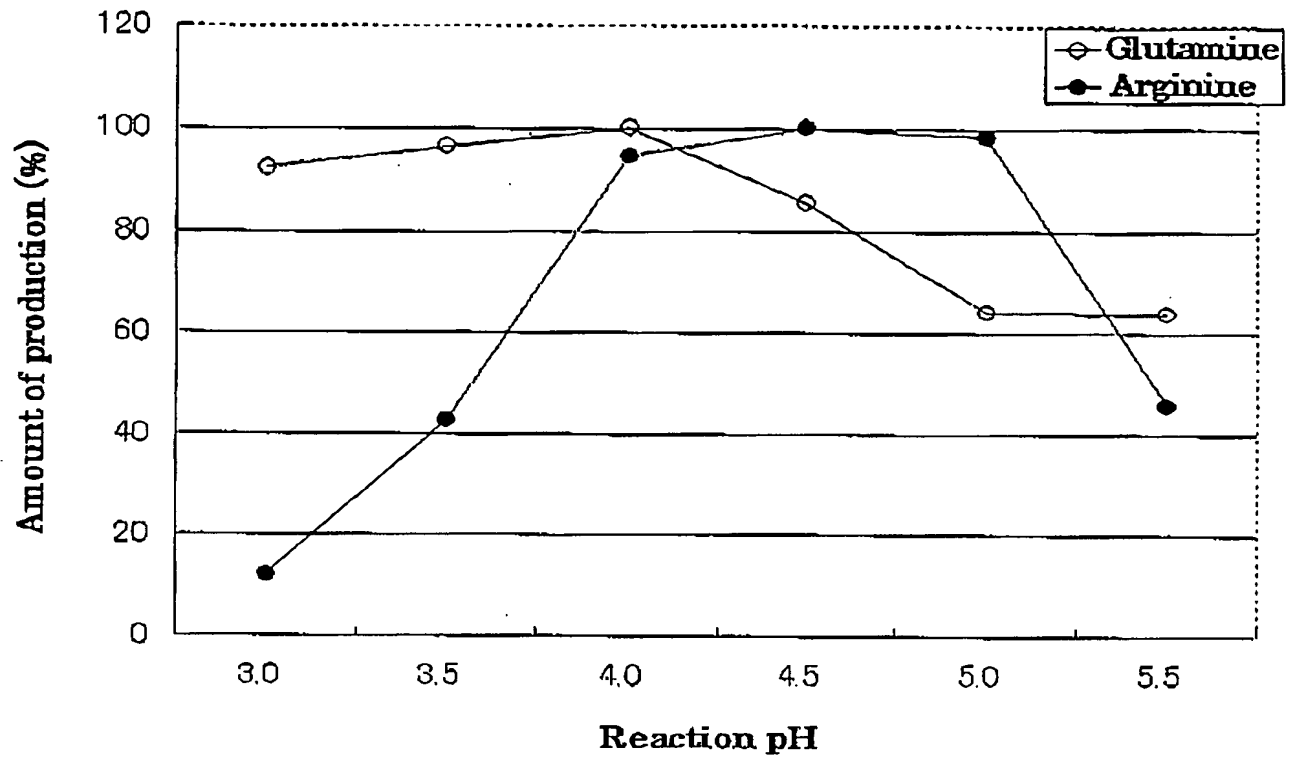
***FIG. 3***

***FIG. 4***

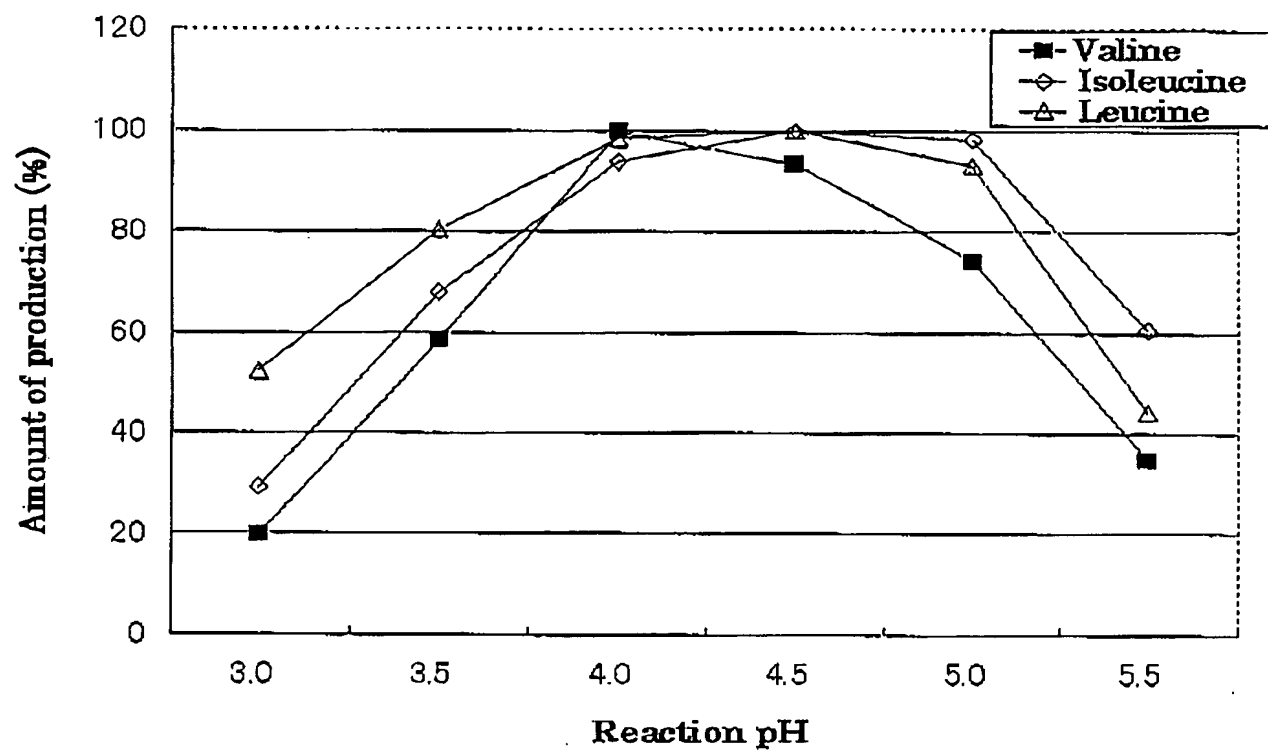
3 / 7

***FIG. 5***

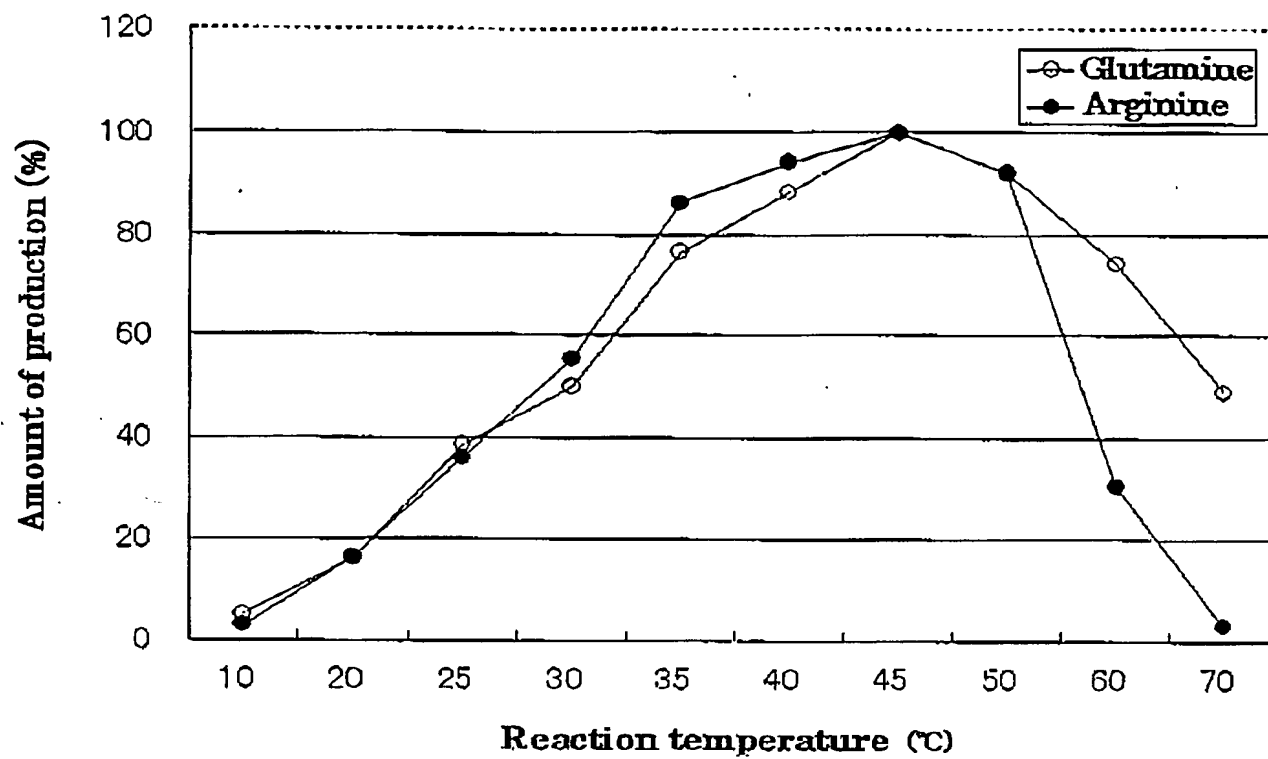
4 / 7

**FIG. 6**

5 / 7

**FIG. 7**

6 / 7

**FIG. 8**

7 / 7

**FIG. 9**